An Update on the Clinicopathologic Features and Pathologic Diagnosis of Hepatitis E in Liver Specimens

Lenggenhager, Daniela ; Weber, Achim

Abstract: Infection with the hepatitis E virus (HEV) is globally seen a leading cause of hepatitis. Now increasingly recognized also in industrialized countries, hepatitis E constitutes a significant health problem worldwide. The patient's immune status determines the clinical course and histopathology of hepatitis E. In immunocompetent patients, hepatitis E usually follows an asymptomatic or subclinical course, but may also present with acute hepatitis. In contrast, immunocompromised patients may develop chronic hepatitis, and patients with preexisting liver diseases are at risk for liver decompensation with potentially fatal outcome. Whereas pathologists only occasionally encounter liver biopsies from immunocompetent individuals with hepatitis E, they are more likely exposed to biopsies from patients with preexisting liver disease or immunocompromised individuals. Histopathologic hallmarks of hepatitis E in immunocompetent patients comprise lobular disarray, lobular, and portal inflammation, as well as hepatocyte necrosis of varying extend and regeneration. Thus, it is similar to acute non-E viral hepatitis, yet further differential diagnoses include autoimmune hepatitis and drug-induced liver injury. Histopathologic findings of hepatitis E in preexisting liver disease are determined by the underlying pathology, but may be more severe. Histopathologic presentation of hepatitis E in immunocompromised patients is highly variable, ranging from minimal active hepatitis to chronic hepatitis with severe activity and progressive fibrosis. Taken together, the variability of the histologic features depending on the clinical context and the overlap with other liver diseases make the histopathologic diagnosis of hepatitis E challenging. Immunohistochemistry for HEV open reading frame 2 protein and molecular testing for HEV RNA are useful tissue-based ancillary tools.

DOI: https://doi.org/10.1097/PAP.0000000000000195

An Update on the Clinicopathologic Features and Pathologic Diagnosis of Hepatitis E in Liver Specimens

Daniela Lenggenhager, MD and Achim Weber, MD

Abstract: Infection with the hepatitis E virus (HEV) is globally seen a leading cause of hepatitis. Now increasingly recognized also in industrialized countries, hepatitis E constitutes a significant health problem worldwide. The patient's immune status determines the clinical course and histopathology of hepatitis E. In immunocompetent patients, hepatitis E usually follows an asymptomatic or subclinical course, but may also present with acute hepatitis. In contrast, immunocompromised patients may develop chronic hepatitis, and patients with preexisting liver diseases are at risk for liver decompensation with potentially fatal outcome. Whereas pathologists only occasionally encounter liver biopsies from immunocompetent individuals with hepatitis E, they are more likely exposed to biopsies from patients with preexisting liver disease or immunocompromised individuals. Histopathologic hallmarks of hepatitis E in immunocompetent patients comprise lobular disarray, lobular, and portal inflammation, as well as hepatocyte necrosis of varying extend and regeneration. Thus, it is similar to acute non–E viral hepatitis, yet further differential diagnoses include autoimmune hepatitis and drug-induced liver injury. Histopathologic findings of hepatitis E in preexisting liver disease are determined by the underlying pathology, but may be more severe. Histopathologic presentation of hepatitis E in immunocompromised patients is highly variable, ranging from minimal active hepatitis to chronic hepatitis with severe activity and progressive fibrosis. Taken together, the variability of the histologic features depending on the clinical context and the overlap with other liver diseases make the histopathologic diagnosis of hepatitis E challenging. Immunohistochemistry for HEV open reading frame 2 protein and molecular testing for HEV RNA are useful tissue-based ancillary tools.

Key Words: liver, hepatitis E virus (HEV), histology, immunohistochemistry, PCR

(*Adv Anat Pathol* 2018;25:273–281)

Since hepatitis E was recognized as a distinct disease in 1980, it was considered an acute, self-limiting, waterborne infection that was largely restricted to developing countries, where it occurred in epidemics and had a dismal prognosis in pregnant women.[1] Nearly a decade ago, it became clear that hepatitis E virus (HEV) infection is more common in industrialized countries than initially thought, with surprisingly high seroprevalence, for example, in the United States of America up to 21%,[2] in the United Kingdom up to 25%,[3] in France on average 30% and up to 86% in a hyperendemic region in the southwest of France.[4,5] In developed countries, HEV is largely transmitted zoonotically by the consumption of uncooked or undercooked meat—in most cases pork or game meat.[6] Although HEV infection in this setting usually takes an asymptomatic and self-limiting course in immunocompetent persons, it can become a serious health risk with possible acute liver failure in patients with preexisting liver disease, and may take a chronic course in immunocompromised patients.[7–10]

HEV is a small, quasienveloped, positive-strand RNA virus of the family *Hepeviridae*, genus *Orthohepevirus*.[11,12] Its 7.2 kb genome has 3 open reading frames (ORF) coding for the viral replicase (ORF1), the capsid protein (ORF2), and a small phosphoprotein required for the secretion of viral particles (ORF3). ORF2 and ORF3 have an intragenotypically and intergenotypically highly conserved, extensively overlapping gene sequence, which make this sequence an ideal target region for molecular testing.[11,13,14] As the ORF2 capsid protein is highly immunogenic, it is the preferred antigen for vaccine development. Probably for the same reason, it also constitutes a strong candidate for the development of tissue-based diagnostic tests, such as immunohistochemistry (IHC).[15,16]

Currently, 5 HEV strains are distinguished which infect humans (Table 1): genotypes (GT) 1, 2, 3, 4, and most recently 7 with variable geographic distribution.[17–21] GT1 and GT2, mainly occur as waterborne epidemic outbreaks in regions such as Asia, Africa, and Central America and seem to be restricted to humans.[21,22] In contrast, the other 3 GTs represent zoonotic infections, mainly transmitted through the consumption of uncooked or undercooked contaminated animal products.[23,24] GT3, occurring worldwide, and GT4, occurring in China and Southeast Asia, have been detected in a wide range of species, such as domestic pigs, game animals, but also bivalves and oysters.[25] Recently GT7 was detected in feces of camel species in the Middle East, North and East Africa, and China, with some limited evidence of HEV GT7 transmission to humans from the consumption of camel meat and milk.[20,26,27]

Clinical manifestation of hepatitis E is highly variable and dependent on the patient's immune status (Table 1). In immunocompetent patients—irrespective of the GT—the majority of HEV infections remain asymptomatic. If clinically apparent, HEV infection in this setting mostly presents as an acute, self-limiting disease, mainly diagnosed by serologic testing. With the exception of pregnant women, acute liver failure due to acute HEV infection in immunocompetent patients may occur, but is extremely rare.[18,28] However, in patients with preexisting liver disease such as (non) alcoholic steatohepatitis, acute decompensation with fatal outcome of HEV infection is more frequently observed.[7] In immunocompromised patients, HEV infection can be easily missed. The usually minimal active hepatitis with no or only unspecific clinical symptoms and only slight and undulating elevation of

From the Department of Pathology and Molecular Pathology, University of Zurich and University Hospital Zurich, Zurich, Switzerland.
Supported by an institutional grant from the center of clinic research (ZKF) innovation foundation to A.W.
The authors have no conflicts of interest to disclose.
Reprints: Achim Weber, MD, Department of Pathology and Molecular Pathology, University Hospital Zurich, Schmelzbergstrasse 12, Zurich CH-8091, Switzerland (e-mail: achim.weber@usz.ch).

TABLE 1. Epidemiology and Clinical Setting of Hepatitis E Virus Infection

	Immunocompetent Patients		Patients With Preexisting Liver Disease	Immunocompromised Patients
	Acute Epidemic Infection	Acute Autochthonous Infection	Acute on Chronic Liver Disease	Chronic-active Hepatitis
Genotypes/ Hosts	GT1, GT2/humans only	GT3, GT4/humans and animals (pigs, game animals, mussels, etc.)	All GT/humans and animals (pigs, game animals, mussels, etc.)	GT3, GT4/humans and animals (pigs, game animals, mussels, etc.) GT7*/camel species (humans)
Global distribution	GT1: Asia, Africa, Central America GT2: mainly Africa and Central America	GT3: worldwide GT4: Southeast Asia, China	Worldwide	GT3: worldwide GT4: Southeast Asia, China GT7: Middle East, North and East Africa, and China
Transmission	Fecally; orally mostly by contaminated drinking water	Mainly consumption of uncooked or undercooked contaminated meat (pork, game, others); rarely blood products	Contaminated drinking water or consumption of uncooked or undercooked contaminated meat (pork, game, others)	Mainly consumption of uncooked or undercooked contaminated meat (pork, game, others)
Patient group	Younger patients; In industrialized countries: travel returnees from endemic regions	Older patients; individuals which consume frequently pork or game meat	Older patients (male > female); frequently with alcohol disease	Any age; solid organ transplant recipients, HIV +patients, patients with hematologic and rheumatologic diseases
Clinical presentation	Ranging from asymptomatic to fulminant	Ranging from asymptomatic to symptomatic	Acute deterioration of a preexisting chronic liver disease	Rarely symptomatic
ALT levels	High ALT levels, usually > 1000 IU/L at time of diagnosis			Usually ALT levels << 1000 IU/L
Clinical course	Usually self-limiting disease with viral clearance; subgroup: pregnant women: higher risk for fatal outcome (particularly in third trimester)	Usually self-limiting disease with viral clearance	Decompensation of the underlying liver disease with high risk of fatal outcome	High failure of viral clearance with possible development of chronification and progressive fibrosis/cirrhosis

*GT7: only 1 case report of chronic hepatitis in a liver-transplanted human being from the United Arab Emirates who consumed regularly camel meat and milk.

ALT indicates alanine aminotransferase; GT, genotype; HIV, human immunodeficiency virus.

transaminases can molder unnoticed, and thus take a chronic course with the development of liver fibrosis.[8–10]

Clinical suspicion of acute hepatitis E can be verified by laboratory tests, either by the detection of HEV-specific IgM antibodies in blood, confirmed by showing IgG antibody levels rising over time, or by molecular detection of HEV RNA, in blood during a relatively short viremic period of about 3 weeks, and in stool samples, in which the virus is detectable for an additional 2-week period.[18,22] In general, antibody-based serological testing for HEV is variably sensitive and specific—especially in immunocompromised patients—and thus remains less robust compared with molecular testing.[29–31] Detection of HEV RNA is not only important for establishing and/or confirming the diagnosis of hepatitis E, but can also be helpful for the monitoring of HEV viremia in case of chronic hepatitis E.

Histopathologic diagnosis of HEV infection is challenging as histologic findings are highly variable and overlap with other causes of hepatitis.[10,32–35] Although hepatitis E diagnosis can be readily made by serologic tests, pathologists are routinely confronted with cases in which serological testing either has not been performed, or results are not yet available. Furthermore, given the comparatively short viremic period, a negative HEV RNA blood test does not definitively exclude a recent infection.

HEPATITIS E VIRUS INFECTION IN IMMUNOCOMPETENT PATIENTS

Acute Epidemic Hepatitis E Virus Infection

Although acute hepatitis E in developing countries is usually a clinical diagnosis, liver histology has been documented in some large epidemic outbreaks, including 1 in Delhi, India in 1955/1956,[36] and 1 in Kashmir in the late 1970.[37] A more recent study based on 11 postmortem biopsies taken from patients with fatal acute fulminant hepatitis E compared the histologic findings with those observed in fulminant hepatitis B.[38] Furthermore, there are occasional case reports on travel-related hepatitis E—for example, following visits to India, Asia, Africa or South America—that comment on histologic changes.[39,40] In summary, the histologic findings described in all these studies are comparable with those found in hepatitis A with either a more inflammatory (so-called "standard" or "classical") pattern, or a more cholestatic (so-called "obstructive") variant. In detail, the following histopathologic changes have been described: a varying degree of mixed portal and lobular inflammation, more or less prominent Kupffer cell infiltrates as well as a variable extent of liver cell necrosis, ranging from single-cell apoptosis to more

TABLE 2. Histologic Findings and Most Important Differential Diagnoses of Hepatitis E Virus Infection

	Immunocompetent Patients	Patients With Preexisting Liver Disease	Immunocompromised Patients
	Acute Epidemic or Autochthonous Infection	**Acute on Chronic Liver Disease**	**Chronic-active Hepatitis**
Histologic features	Acute hepatitis mixed lobular and portal inflammation, interface activity Lobular disarray, rosettes' formation and ballooned hepatocytes Increased number of Kupffer cells Variable degree of hepatocyte necrosis (mostly spotty necrosis, but bridging necrosis or only single apoptotic hepatocytes also possible) Expanded portal tracts with mixed inflammation cholangitis and ductular reaction Hepatocytic and canalicular bilirubinostasis Subgroup: fulminant liver failure panlobular necrosis parenchymal collaps bilirubinostasis clearing by ceroid-laden macrophages	Cirrhosis Ballooned hepatocytes +/−Mallory-Denk hyaline necroinflammatory infiltrates Kupffer cell aggregates Siderosis variable degree of hepatocyte necrosis Cholangitis cholestasis/bilirubinostasis	Portal-based mononuclear inflammation (predominantly lymphocytes) Lacking or low interface activity Variable degree of hepatocyte necrosis (mostly single scattered apoptotic hepatocytes) Fibrosis development
Most important differential diagnoses	Non–E hepatotropic viruses (eg, HAV) Nonhepatotropic viruses (eg, EBV) autoimmune hepatitis drug-induced liver injury and toxins Subgroup: fulminant liver failure Drug-induced liver injury and toxins Non–E hepatotropic viruses (eg, HBV) nonhepatotropic viruses (eg, adenovirus) Autoimmune hepatitis Metabolic diseases (eg, Wilsons' disease)	Decompensation of underlying chronic liver disease, for example, ASH or NASH	Non–E hepatotropic viruses (HBV, HCV) autoimmune hepatitis In liver transplant patients: acute cellular rejection recurrence of initial viral hepatitis (eg, HCV, HBV) drug-induced changes by immunosuppressive therapy itself

ASH indicates alcoholic steatohepatitis; HAV, hepatitis A virus; HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis.

severe forms with bridging and subtotal hepatic necrosis. In the more cholestatic variants, ballooning degeneration, rosettes' formation as well as hepatocellular and intracanalicular bilirubinostasis were also frequently observed. In severe cases with bridging or (sub)total necrosis and progressive ductular proliferation, even bile plugs within dilated cholangioles have been reported. The most important differential diagnoses regarding the pattern of acute (cholestatic) hepatitis are non–E viral infections (eg, hepatitis A virus, EBV), autoimmune hepatitis, and drug-induced liver injury (Table 2). Pathologists in industrialized countries are not only confronted with liver biopsies performed on travel-related cases of hepatitis E, but also with liver explants in the setting of liver transplantation due to rare cases of acute liver failure following imported HEV infection, in particular in young women.[28,34,39] Liver explants usually are small and lightweight due to parenchymal collapse following panlobular necrosis. Typical histopathologic features are bilirubinostasis, massive clearing by ceroid-laden macrophages, and extensive ductular reaction reflecting futile regeneration. In such a setting, the most important differential diagnoses are drug-induced liver injury and toxins, infections with non–E viruses (eg, HBV, adenovirus) as well as autoimmune hepatitis and metabolic diseases such as Wilsons' disease (Table 2).

Histologic findings are illustrated in Figure 1 by 2 cases of travel-related hepatitis E of varying severity, both HEV GT 1 infections and occurring after a trip to India: Figure 1A shows the liver biopsy of a 50-year-old man who developed acute self-limiting hepatitis E with lobular disarray as well as portal and lobular inflammation. IHC for HEV ORF 2 protein displayed cytoplasmic and nuclear positivity in scattered hepatocytes as shown in the insert.[39] Figure 1B illustrates the histologic findings in a liver explant from a 26-year-old female patient who developed fulminant liver failure with panlobular necrosis, parenchymal collapse, and ducutular proliferation as a sign of futile regeneration as well as some portal inflammation. Whereas HEV was still detectable by tissue-based RT-PCR,[34] immunohistochemical detection using a HEV ORF 2 antibody was not possible in this subtotally necrotic tissue.[16]

Acute Autochthonous Hepatitis E Virus Infection

Although the awareness of autochthonous HEV infection—mostly with GT3—occurring in industrialized countries has increased during the last 10 years, reports on histologic features of acute autochthonous HEV infection in immunocompetent patients are still rather rare, mainly because the diagnosis is usually based on serology. If a liver biopsy is performed, this is usually done early in the diagnostic process, when the list of differential diagnoses is still long, and results of serological testing are not yet available. Histopathologic findings of acute autochthonous HEV infection with GT3 are similar to those described in acute hepatitis with HEV GT1. Immunocompetent individuals usually show a prominent lobular disarray with rosettes'

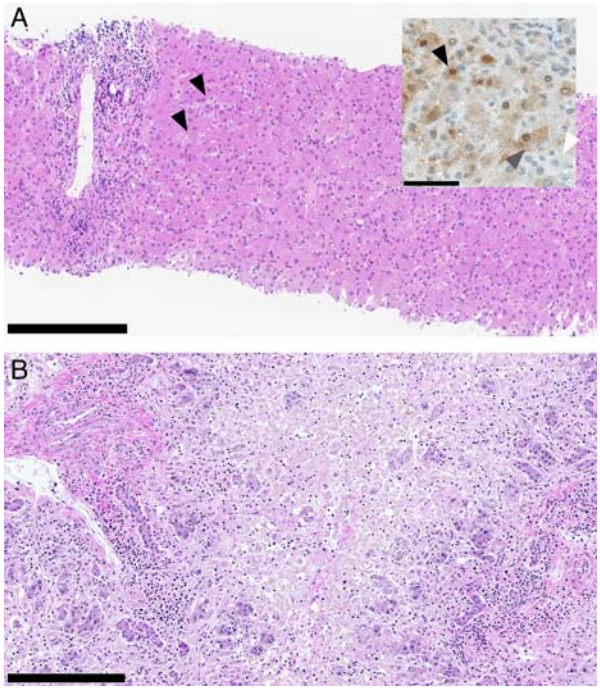

FIGURE 1. Histopathology of endemic (travel-related) hepatitis E (HEV genotype 1 infection), both cases occurring following a trip to India. A, Liver biopsy of a male patient with acute self-limiting hepatitis E. Note lobular disarray, portal, and lobular inflammation as well as Kupffer cell hyperplasia (black arrowheads). Scale bar: 250 μm. Insert: immunohistochemistry for HEV open reading frame 2 protein displaying hepatocytes with predominately nuclear reactivity (black arrowhead), predominately cytoplasmic reactivity (gray arrowhead), and no reactivity (white arrowhead). Scale bar: 50 μm. B, Liver explant of a young female patient with travel-related acute hepatitis E resulting in fulminant liver failure. Note panlobular necrosis, predominantly periportal inflammation, and futile regeneration. Scale bar: 250 μm. HEV indicates hepatitis E virus.

formation and a variable number of ballooned hepatocytes as well as a variable degree of necrosis, ranging from usually spotty necrosis to pericentral and even panlobular necrosis.[32–35,41] Portal tracts are usually considerably expanded with bile duct proliferations and a mixed inflammatory cell infiltrate comprising lymphocytes, plasma cells, histiocytes, and also many neutrophils and eosinophils, which can involve the bile duct epithelium.[32,41] Malcolm and colleagues describe a variable degree of hepatocellular and canalicular bilirubinostasis and a zonal distribution pattern of inflammatory cells in the portal tracts. At the periphery/interface they observed more polymorphs, while a lymphohistiocytic component (including plasma cells) was more pronounced in the center of the portal tract.[41] Drebber et al[33] pointed in addition at the absence of fibrosis, which is a feature of chronic hepatitis. Thus, the differential diagnosis of an acute autochthonous HEV infection in immunocompetent patients is basically the same as for acute epidemic HEV infection (Table 2).

Histologic findings of a typical acute autochthonous HEV GT3 infection in an immunocompetent patient are illustrated in Figures 2A–C. The liver biopsy of a 67-year-old female patient with acute self-limiting hepatitis displays lobular disarray as well as portal and lobular inflammation. Besides scattered apoptotic hepatocytes, many prominent macrophages/activated Kupffer cells are distinguishable, even focally clustered, indicating extensive recent piecemeal necrosis. No signs of underlying chronic liver disease were observable.

HEPATITIS E VIRUS INFECTION IN PATIENTS WITH PREEXISTING CHRONIC LIVER DISEASES

Patients, with preexisting (chronic) liver disease who are infected with HEV, are prone to develop acute on chronic liver failure. Already a decade ago, this unfavorable course has been well documented for HEV infections occurring in both, developing and industrialized countries.[7,32,42] In a large study from India, a panoply of different underlying chronic liver diseases has been identified: from chronic HBV and hepatitis C virus infections over alcoholic liver disease to metabolic diseases such as Wilsons' disease and hemochromatosis, and even perfusion-related liver diseases.[43] In industrialized countries, patients with cirrhosis due to alcoholic and eventually nonalcoholic steatohepatitis may represent the largest patient groups that develop a rapid decompensation on autochthonous HEV infection.[40,44–46] Furthermore, it seems that cirrhotic patients are prone to be infected with HEV, but whether overconsumption of alcohol in itself is associated with increased susceptibility to acute hepatitis E is still unclear.[47]

Histopathologic findings of HEV infection in this setting reveal acute damage in addition to an underlying fibrotic/cirrhotic process. Histologic findings in the majority of the above-mentioned studies are described as follows: liver parenchyma with advanced fibrosis or cirrhosis, marked necroinflammatory activity with ballooned hepatocytes with or without Mallory-Denk hyaline, surrounded by neutrophils, Kupffer cell aggregates with siderosis, scattered apoptotic hepatocytes, but also confluent necrosis as well as prominent acute cholangitis and cholestasis. All those histologic features are nonspecific, and in the context of an established alcohol-related cirrhosis, they can easily be mistaken for acute alcoholic hepatitis.[48] As the diagnosis of acute hepatitis E infection in patients with chronic liver disease is often overlooked, it is important for pathologists as well as for clinicians to have HEV infection on their list of differential diagnoses, as an early diagnosis can prompt early therapy with the antiviral substance ribavirin.[49]

Histologic findings of an acute autochthonous HEV GT3 infection in a patient with preexisting chronic liver disease are illustrated in Figures 2D–F. The liver biopsy of a 66-year-old female patient with cirrhosis on nonalcoholic steatohepatitis shows nodular liver parenchyma with rosettes' formation and steatosis, surrounded by broad fibrotic septae. The hepatocytes are ballooned with Mallory-Denk hyaline in the cytoplasm.

HEPATITIS E VIRUS INFECTION IN IMMUNOCOMPROMISED PATIENTS

Following a seminal study from France published in 2008,[8] there were several papers reporting that HEV—mostly GT3—can cause chronic infections in immunocompromised patients with significant morbidity and mortality. The studies comprised mostly solid organ transplant recipients,[8,50–53] and human immunodeficiency virus-positive patients,[3,54] but also included patients with hematological malignancies receiving chemotherapy or stem cell transplantation,[55,56] and patients treated with immunosuppressive drugs for other, for example, rheumatologic

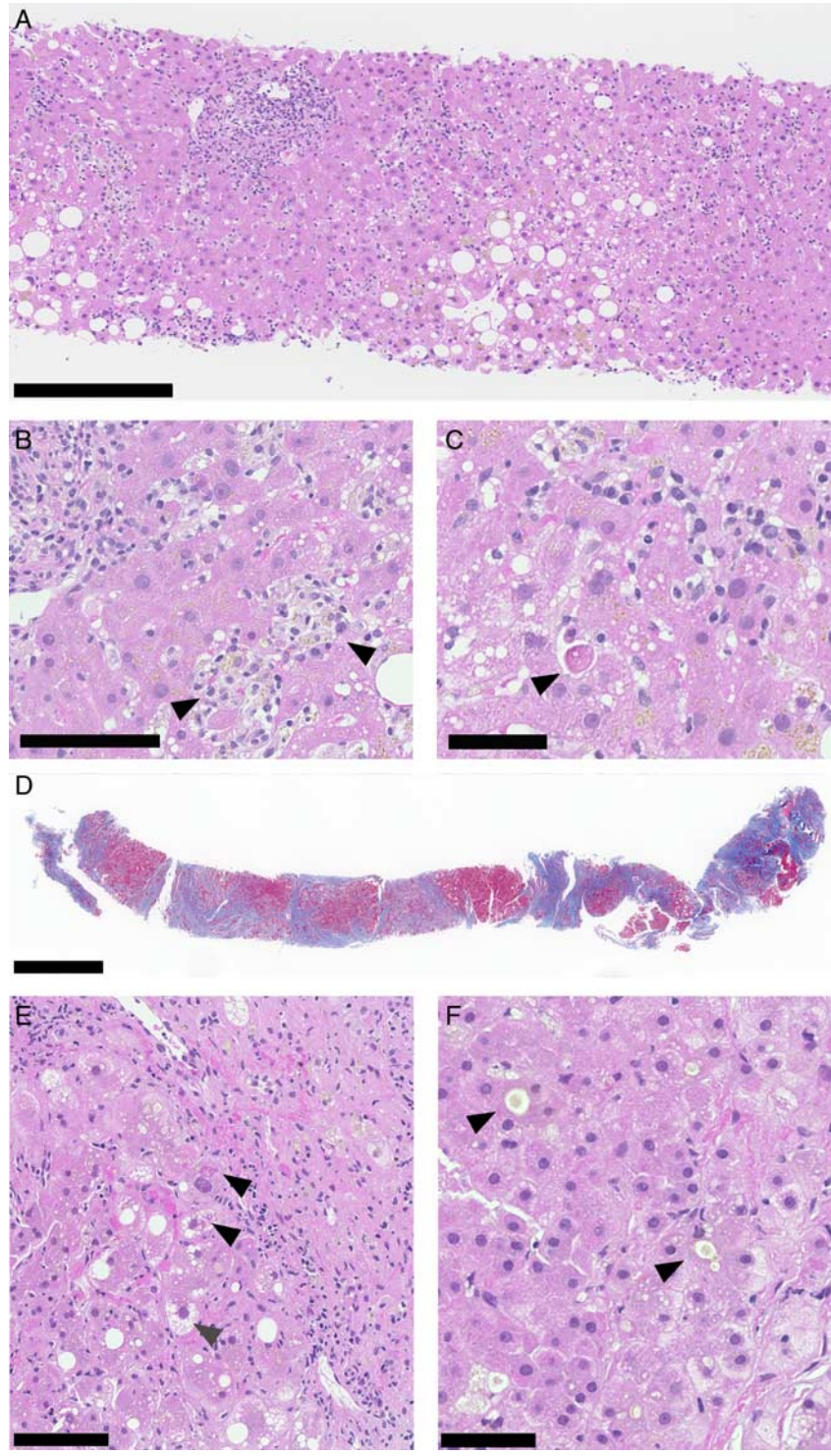

FIGURE 2. A–F, Histopathology of autochthonous hepatitis E (HEV genotype 3 infection). A–C Case of an immunocompetent individual. A, Liver biopsy of a female patient with acute self-limiting hepatitis E. Note lobular disarray as well as portal and lobular inflammation. Scale bar: 250 μm. B, Lobular inflammation and increased number of prominent pigmented Kupffer cells (black arrowheads). Scale bar: 100 μm. C, Scattered hepatocyte apoptosis (black arrowhead). Scale bar: 50 μm. D–F, Case of a patient with underlying chronic liver disease. D, Liver biopsy of a female patient with cirrhosis due to nonalcoholic steatohepatitis and additional acute hepatitis E. Low power view revealing broad bridging fibrotic septae, parenchymal nodularity and steatosis. Masson trichrome stain. Scale bar: 1 mm. E, Hepatocytes with ballooning (gray arrowhead) and Mallory-Denk hyaline (black arrowhead). Scale bar: 100 μm. F, Rosettes formation (black arrowheads). Sale bar: 50 μm. HEV indicates hepatitis E virus.

diseases.[56] The risk of chronification of HEV infection in solid organ transplant recipients may depend on the immunosuppressive drug. Thus, the percentage reported in different studies ranges considerably, between 21% and 66%.[9] In this clinical setting, untreated chronic HEV hepatitis can progress rapidly to cirrhosis, as reported by several of the above-mentioned studies. Recently, it was suggested that in the setting of organ transplantation, chronic HEV infection should be considered when HEV replication persists for >3 months. This concept is in contrast to the usual definition of chronic hepatitis (virus replication of >6 months), as in this particular patient group no spontaneous clearance of HEV was observed between 3 and 6 months after an acute infection.[57] As the differential diagnosis of elevated liver enzymes in immunocompromised patients is broad, the threshold to perform a liver biopsy is rather low. Especially in liver-transplanted patients, acute cellular rejection has to be evaluated histologically. Liver damage in immunocompromised patients has been reported to be generally less acute and less extensive than in immunocompetent individuals, with often a subclinical/anicteric course and only a slight undulating elevation of transaminases over time.[8,35,50,58,59] This also explains why HEV infection in this patient group is easily overlooked for weeks or even months.

Histologic findings in chronic-active HEV infections are similar to those observed in chronic-active viral hepatitis B or C. They include lobular activity with variable degree of piecemeal necrosis, mainly presenting as single-cell apoptosis without significant inflammation or hepatocytes' ballooning, portal lymphocytic infiltrates of variable degree without significant interface activity and eventually development of fibrosis.[8,50] In our own cases, we noticed a generally lower degree of lobular disarray and a lack of cholestasis or bilirubinostasis, again indicative of less extensive damage.[10] Development of fibrosis has been documented in detail in one of our patients who had developed HEV infection after liver transplantation for cirrhosis due to alpha-1-antitypsin deficiency. Over a period of >3 years, he developed progressive liver fibrosis which was documented in a series of 5 liver biopsies. The same case also illustrates how variable the inflammatory activity can be during HEV infection, ranging from nearly nonreactive to prominent inflammatory activity.[10] In immunocompromised patients, the most important differential diagnoses are other viral infections such as HBV and hepatitis C virus infection, as well as autoimmune hepatitis. In liver transplant recipients, the most important differential diagnoses are acute cellular rejection and newly acquired or recurrent non–E viral hepatitis (Table 2). Furthermore, histopathologic findings can also be mistaken to be related to the immunosuppressive therapy itself.[8]

Histologic findings at different timepoints of an autochthonous HEV GT3 infection after liver transplantation in a 66-year-old patient are illustrated in Figure 3. The liver biopsy taken almost 3 years after liver transplantation showed moderate portal and lobular inflammation with some lobular disarray and numerous apoptotic hepatocytes. Furthermore, portal, periportal, and pericentral fibrosis was detectable by Sirius red staining. However, the liver biopsy taken in an early phase after HEV infection displayed unremarkable portal tracts with lack of inflammatory infiltrates, and only few scattered apoptotic hepatocytes.[10] HEV ORF 2 protein IHC can be very helpful, as illustrated in another case.

ANCILLARY TOOLS FOR THE HISTOPATHOLOGIC DIAGNOSIS OF HEPATITIS E

As outlined above, histopathologic features of HEV infection are nonspecific and overlap substantially with those of hepatitis of other etiology. Thus, ancillary tools are required for making a definitive histopathologic diagnosis. Reliable diagnostic tools in the hands of pathologists are still polymerase chain reaction-(PCR) based molecular tests, targeting the intragenotypically and intergenotypically highly conserved ORF 2 and 3 gene region. By modifying those tests for the detection of HEV RNA in formalin-fixed paraffin-embedded liver tissues, they have been successfully applied to liver specimens by several pathology laboratories, including ourselves.[10,33–35] However, PCR methods are not available in all pathology laboratories.

Naturally, in situ techniques such as in situ hybridization (ISH) and IHC, are convenient ancillary tools for pathologists. Recently, the literature about in situ localization of HEV in liver tissues has been growing. Until 2016, only a few studies on the detection of HEV RNA and HEV proteins in the liver—including primates' and swine liver as well as humanized mice—were reported.[60–64] In a recent study, Prost et al[35] performed HEV ISH in a substantial collective of human liver biopsies. They concluded that in situ RNA testing is very specific, but less sensitive (61%) compared with tissue-based PCR testing (91%). However, depending on the clinical setting and the equipment of a pathology laboratory, ISH can have its diagnostic utility. In our own study on in situ localization of HEV in the human liver, a HEV antibody against the capsid protein ORF 2 showed the most reliable results with a high specificity rate and a sensitivity rate of 70%, detecting 82% of the cases tested positive by a tissue-based molecular PCR method.[65] As we found ORF 2 IHC to be more sensitive and robust in cases of chronic-active hepatitis E, it can represent a valuable ancillary tool for pathologists dealing with liver biopsies from immunocompromised patients. Last but not least, IHC methods are low cost and have a shorter turnaround time.

As illustrated in Figures 1 and 3, the ORF 2 antibody displayed a different extent of immunohistochemical positivity depending on the immune status of the patient. Although liver biopsies from immunocompetent individuals generally showed fewer, and in some cases only single scattered positive hepatocytes, those from immunocompromised patients usually displayed larger and often confluent areas of positive hepatocytes. In addition to already previously described cytoplasmic and canalicular staining patterns of ORF 2,[64,66] we could detect a previously nondescribed nuclear staining pattern in our study, which indicates that this capsid protein may interact with nuclear components.

In summary, ancillary tools for tissue-based HEV diagnostics available nowadays are applicable in daily routine diagnostics.

EXTRAHEPATIC MANIFESTATIONS OF HEPATITIS E VIRUS INFECTION

Finally, although the HEV is primarily hepatotropic, it is well documented that it also infects nonhepatocytic cells, and extrahepatic manifestations of HEV are increasingly being recognized.[67] These might be caused directly or indirectly, that is, immune-mediated, and are extensively described for neurological manifestations such as neuralgic amyotrophy, neuritis, and Guillain-Barré syndrome,[68–71] but also include hematological abnormalities including lymphocytosis or lymphopenia, and thrombocytopenia.[70,72]

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

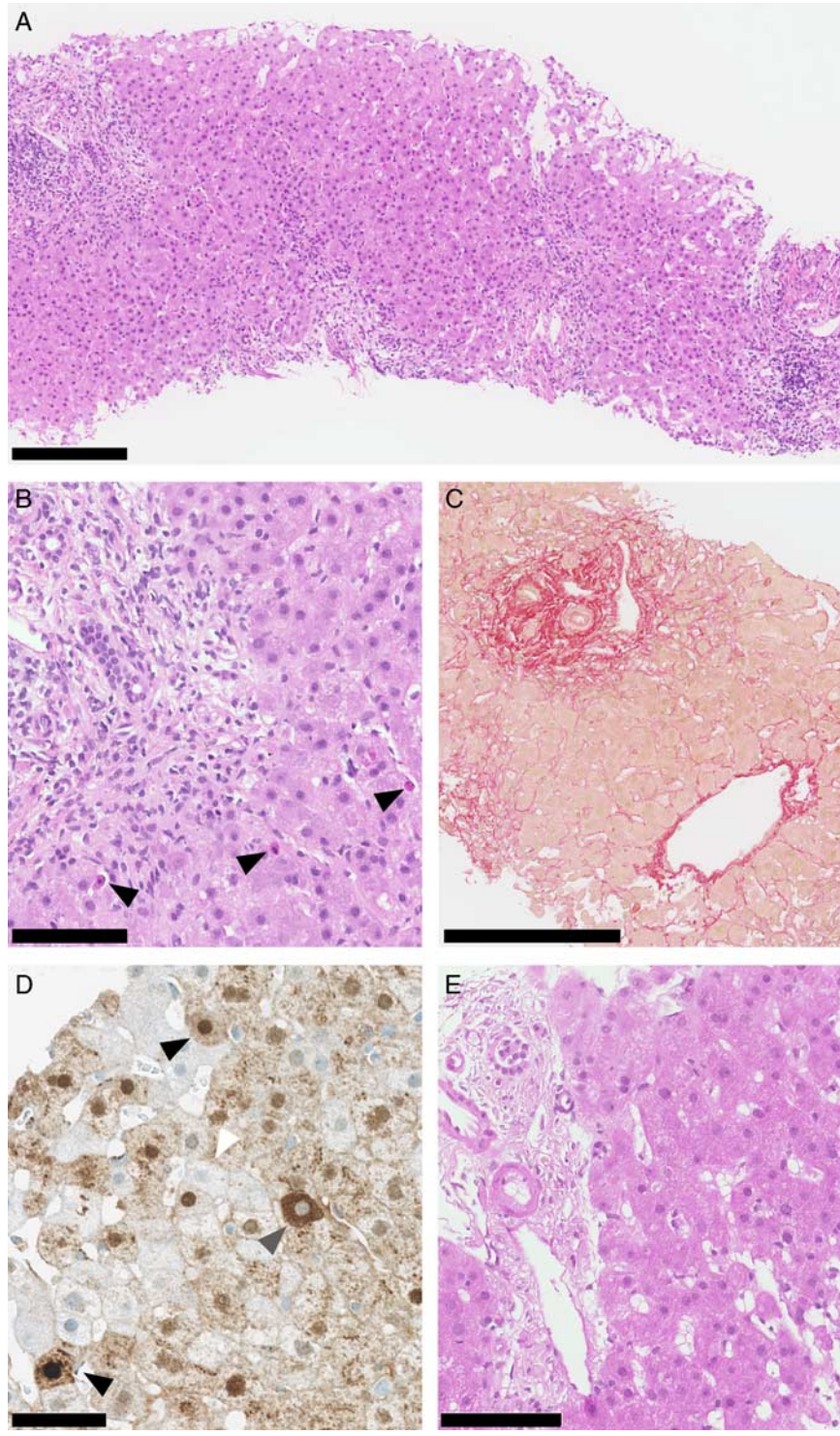

FIGURE 3. Histopathology of autochthonous hepatitis E (HEV genotype 3 infection) in immunocompromised individuals. A, Liver biopsy of a male patient with chronic hepatitis E (HEV genotype 3 infection) after liver transplantation. Note portal and lobular inflammation and some lobular disarray. Scale bar: 250 μm. B, High power view displaying predominantly portal inflammation and piecemeal necrosis with numerous apoptotic hepatocytes (black arrowheads). Scale bar: 100 μm. C, Portal, periportal, and pericentral fibrosis. Sirius red stain, scale bar: 250 μm. D, Immunohistochemistry for HEV open reading frame 2 protein displaying widespread positivity in hepatocytes with predominately nuclear reactivity (black arrowhead), predominately cytoplasmic reactivity (gray arrowhead), and no reactivity (white arrowhead). Scale bar: 50 μm. E, Liver biopsy taken in an early phase after infection with HEV. Note unremarkable portal tract with lack of inflammation. Scale bar: 100 μm. HEV indicates hepatitis E virus.

SUMMARY AND OUTLOOK

During the last decades, we witnessed a paradigm shift for hepatitis E which is no longer regarded as a disease restricted to resources-poor countries, but rather a globally relevant health problem.[73,74] That hepatitis E has been underreported or misdiagnosed in the past remains without doubt; however, the awareness for the disease has recently increased in Europe.[5,75] Taking into account the evidence of hepatitis E in the past,[76] and conflicting epidemiologic data,[2,77,78] it cannot be excluded that the perception of hepatitis E soon will change similarly in the United States. Recent progress in the development of experimental systems for hepatitis E,[63,64] along with novel molecular findings on HEV,[14,21] brought not only insight into basic mechanisms of this virus, but provided also the rational for the development of ancillary, tissue-based diagnostic tools.[16] Given that the above outlined broad spectrum of hepatitis E histopathologic patterns significantly overlaps with that of hepatitis of other etiology, in particular non–E viral hepatitis, autoimmune hepatitis, and drug-induced liver injury, and is determined by host factors, we expect hepatitis E to remain a challenging histopathologic diagnosis in the near future. However, increased awareness of hepatitis E and a low threshold for applying ancillary tools, that is, HEV RNA testing and/or IHC for HEV ORF 2 protein, will enable pathologists to reliably and timely reach the correct diagnosis.

REFERENCES

1. Khuroo MS, Teli MR, Skidmore S, et al. Incidence and severity of viral hepatitis in pregnancy. *Am J Med*. 1981;70:252–255.
2. Kuniholm MH, Purcell RH, McQuillan GM, et al. Epidemiology of hepatitis E virus in the United States: results from the Third National Health and Nutrition Examination Survey, 1988-1994. *J Infect Dis*. 2009;200:48–56.
3. Dalton HR, Bendall RP, Keane FE, et al. Persistent carriage of hepatitis E virus in patients with HIV infection. *N Engl J Med*. 2009;361:1025–1027.
4. Mansuy JM, Gallian P, Dimeglio C, et al. A nationwide survey of hepatitis E viral infection in French blood donors. *Hepatology*. 2016;63:1145–1154.
5. Hartl J, Otto B, Madden RG, et al. Hepatitis E seroprevalence in Europe: a meta-analysis. *Viruses*. 2016;8:211–224.
6. Clemente-Casares P, Ramos-Romero C, Ramirez-Gonzalez E, et al. Hepatitis E virus in industrialized countries: the silent threat. *Biomed Res Int*. 2016;2016:9838041.
7. Kumar Acharya S, Kumar Sharma P, Singh R, et al. Hepatitis E virus (HEV) infection in patients with cirrhosis is associated with rapid decompensation and death. *J Hepatol*. 2007;46:387–394.
8. Kamar N, Selves J, Mansuy JM, et al. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. *N Engl J Med*. 2008;358:811–817.
9. Kamar N, Garrouste C, Haagsma EB, et al. Factors associated with chronic hepatitis in patients with hepatitis E virus infection who have received solid organ transplants. *Gastroenterology*. 2011;140:1481–1489.
10. Protzer U, Bohm F, Longerich T, et al. Molecular detection of hepatitis E virus (HEV) in liver biopsies after liver transplantation. *Mod Pathol*. 2015;28:523–532.
11. International Committee on Taxonomy of Viruses Hepeviridae Study Group, Smith DB, Simmonds P, Jameel S, et al. Consensus proposals for classification of the family Hepeviridae. *J Gen Virol*. 2014;95:2223–2232.
12. Feng Z, Hirai-Yuki A, McKnight KL, et al. Naked viruses that aren't always naked: quasi-enveloped agents of acute hepatitis. *Annu Rev Virol*. 2014;1:539–560.
13. Huang S, Zhang X, Jiang H, et al. Profile of acute infectious markers in sporadic hepatitis E. *PLoS One*. 2010;5:e13560.
14. Debing Y, Moradpour D, Neyts J, et al. Update on hepatitis E virology: implications for clinical practice. *J Hepatol*. 2016;65:200–212.
15. Purcell RH, Emerson SU. Hepatitis E: an emerging awareness of an old disease. *J Hepatol*. 2008;48:494–503.
16. Lenggenhager D, Gouttenoire J, Malehmir M, et al. Visualization of hepatitis E virus RNA and proteins in the human liver. *J Hepatol*. 2017;67:471–479.
17. Aggarwal R, Jameel S. Hepatitis E. *Hepatology*. 2011;54:2218–2226.
18. Kamar N, Bendall R, Legrand-Abravanel F, et al. Hepatitis E. *Lancet*. 2012;379:2477–2488.
19. Meng XJ. Expanding host range and cross-species infection of hepatitis E virus. *PLoS Pathog*. 2016;12:e1005695.
20. Woo PC, Lau SK, Teng JL, et al. New hepatitis E virus genotype in camels, the Middle East. *Emerg Infect Dis*. 2014;20:1044–1048.
21. Nimgaonkar I, Ding Q, Schwartz RE, et al. Hepatitis E virus: advances and challenges. *Nat Rev Gastroenterol Hepatol*. 2018;15:96–110.
22. Hoofnagle JH, Nelson KE, Purcell RH. Hepatitis E. *N Engl J Med*. 2012;367:1237–1244.
23. Legrand-Abravanel F, Mansuy JM, Dubois M, et al. Hepatitis E virus genotype 3 diversity, France. *Emerg Infect Dis*. 2009;15:110–114.
24. Meng XJ. Zoonotic and foodborne transmission of hepatitis E virus. *Semin Liver Dis*. 2013;33:41–49.
25. Grodzki M, Schaeffer J, Piquet JC, et al. Bioaccumulation efficiency, tissue distribution, and environmental occurrence of hepatitis E virus in bivalve shellfish from France. *Appl Environ Microbiol*. 2014;80:4269–4276.
26. Rasche A, Saqib M, Liljander AM, et al. Hepatitis E virus infection in dromedaries, North and East Africa, United Arab Emirates, and Pakistan, 1983-2015. *Emerg Infect Dis*. 2016;22:1249–1252.
27. Lee GH, Tan BH, Teo EC, et al. Chronic infection with camelid hepatitis E virus in a liver transplant recipient who regularly consumes camel meat and milk. *Gastroenterology*. 2016;150:355.e353–357.e353.
28. Chris RB, Keystone JS. Fulminant hepatic failure from hepatitis E in a non-pregnant female traveller. *J Travel Med*. 2016;23:1–3.
29. El-Sayed Zaki M, El-Deen Zaghloul MH, El Sayed O. Acute sporadic hepatitis E in children: diagnostic relevance of specific immunoglobulin M and immunoglobulin G compared with nested reverse transcriptase PCR. *FEMS Immunol Med Microbiol*. 2006;48:16–20.
30. Gyarmati P, Mohammed N, Norder H, et al. Universal detection of hepatitis E virus by two real-time PCR assays: TaqMan and Primer-Probe Energy Transfer. *J Virol Methods*. 2007;146:226–235.
31. Khudyakov Y, Kamili S. Serological diagnostics of hepatitis E virus infection. *Virus Res*. 2011;161:84–92.
32. Peron JM, Danjoux M, Kamar N, et al. Liver histology in patients with sporadic acute hepatitis E: a study of 11 patients from South-West France. *Virchows Arch*. 2007;450:405–410.
33. Drebber U, Odenthal M, Aberle SW, et al. Hepatitis E in liver biopsies from patients with acute hepatitis of clinically unexplained origin. *Front Physiol*. 2013;4:351.
34. Chijioke O, Bawohl M, Springer E, et al. Hepatitis e virus detection in liver tissue from patients with suspected drug-induced liver injury. *Front Med (Lausanne)*. 2015;2:20.
35. Prost S, Crossan CL, Dalton HR, et al. Detection of viral hepatitis E in clinical liver biopsies. *Histopathology*. 2017;71:580–590.
36. Gupta DN, Smetana HF. The histopathology of viral hepatitis as seen in the Delhi epidemic (1955-56). *Indian J Med Res*. 1957;45:101–113.
37. Khuroo MS. Study of an epidemic of non-A, non-B hepatitis. Possibility of another human hepatitis virus distinct from post-transfusion non-A, non-B type. *Am J Med*. 1980;68:818–824.

38. Agrawal V, Goel A, Rawat A, et al. Histological and immunohistochemical features in fatal acute fulminant hepatitis E. *Indian J Pathol Microbiol*. 2012;55:22–27.
39. Friedman LS, Lee SR, Nelson SB, et al. Case 36-2016. A 50-year-old man with acute liver injury. *N Engl J Med*. 2016;375: 2082–2092.
40. Crossan CL, Simpson KJ, Craig DG, et al. Hepatitis E virus in patients with acute severe liver injury. *World J Hepatol*. 2014;6:426–434.
41. Malcolm P, Dalton H, Hussaini HS, et al. The histology of acute autochthonous hepatitis E virus infection. *Histopathology*. 2007;51:190–194.
42. Dalton HR, Bendall R, Ijaz S, et al. Hepatitis E: an emerging infection in developed countries. *Lancet Infect Dis*. 2008;8:698–709.
43. Radha Krishna Y, Saraswat VA, Das K, et al. Clinical features and predictors of outcome in acute hepatitis A and hepatitis E virus hepatitis on cirrhosis. *Liver Int*. 2009;29:392–398.
44. Peron JM, Bureau C, Poirson H, et al. Fulminant liver failure from acute autochthonous hepatitis E in France: description of seven patients with acute hepatitis E and encephalopathy. *J Viral Hepat*. 2007;14:298–303.
45. Lockwood GL, Fernandez-Barredo S, Bendall R, et al. Hepatitis E autochthonous infection in chronic liver disease. *Eur J Gastroenterol Hepatol*. 2008;20:800–803.
46. Inagaki Y, Oshiro Y, Hasegawa N, et al. Clinical features of hepatitis E virus infection in Ibaraki, Japan: autochthonous hepatitis E and acute-on-chronic liver failure. *Tohoku J Exp Med*. 2015;235:275–282.
47. Xu B, Yu HB, Hui W, et al. Clinical features and risk factors of acute hepatitis E with severe jaundice. *World J Gastroenterol*. 2012;18:7279–7284.
48. Dalton HR. Hepatitis: hepatitis E and decompensated chronic liver disease. *Nat Rev Gastroenterol Hepatol*. 2012;9:430–432.
49. Goyal R, Kumar A, Panda SK, et al. Ribavirin therapy for hepatitis E virus-induced acute on chronic liver failure: a preliminary report. *Antivir Ther*. 2012;17:1091–1096.
50. Haagsma EB, Niesters HG, van den Berg AP, et al. Prevalence of hepatitis E virus infection in liver transplant recipients. *Liver Transpl*. 2009;15:1225–1228.
51. Gerolami R, Moal V, Colson P. Chronic hepatitis E with cirrhosis in a kidney-transplant recipient. *N Engl J Med*. 2008;358: 859–860.
52. Pischke S, Suneetha PV, Baechlein C, et al. Hepatitis E virus infection as a cause of graft hepatitis in liver transplant recipients. *Liver Transpl*. 2010;16:74–82.
53. Koning L, Pas SD, de Man RA, et al. Clinical implications of chronic hepatitis E virus infection in heart transplant recipients. *J Heart Lung Transplant*. 2013;32:78–85.
54. Shrestha A, Adhikari A, Bhattarai M, et al. Prevalence and risk of hepatitis E virus infection in the HIV population of Nepal. *Virol J*. 2017;14:228.
55. Ollier L, Tieulie N, Sanderson F, et al. Chronic hepatitis after hepatitis E virus infection in a patient with non-Hodgkin lymphoma taking rituximab. *Ann Intern Med*. 2009;150:430–431.
56. Honer zu Siederdissen C, Pischke S, Schlue J, et al. Chronic hepatitis E virus infection beyond transplantation or human immunodeficiency virus infection. *Hepatology*. 2014;60: 1112–1113.
57. Kamar N, Rostaing L, Legrand-Abravanel F, et al. How should hepatitis E virus infection be defined in organ-transplant recipients? *Am J Transplant*. 2013;13:1935–1936.
58. Legrand-Abravanel F, Kamar N, Sandres-Saune K, et al. Characteristics of autochthonous hepatitis E virus infection in solid-organ transplant recipients in France. *J Infect Dis*. 2010;202: 835–844.
59. Kamar N, Abravanel F, Selves J, et al. Influence of immunosuppressive therapy on the natural history of genotype 3 hepatitis-E virus infection after organ transplantation. *Transplantation*. 2010;89:353–360.
60. Lau JY, Sallie R, Fang JW, et al. Detection of hepatitis E virus genome and gene products in two patients with fulminant hepatitis E. *J Hepatol*. 1995;22:605–610.
61. Choi C, Chae C. Localization of swine hepatitis E virus in liver and extrahepatic tissues from naturally infected pigs by in situ hybridization. *J Hepatol*. 2003;38:827–832.
62. Ha SK, Chae C. Immunohistochemistry for the detection of swine hepatitis E virus in the liver. *J Viral Hepat*. 2004;11:263–267.
63. Sayed IM, Verhoye L, Cocquerel L, et al. Study of hepatitis E virus infection of genotype 1 and 3 in mice with humanised liver. *Gut*. 2017;66:920–929.
64. Allweiss L, Gass S, Giersch K, et al. Human liver chimeric mice as a new model of chronic hepatitis E virus infection and preclinical drug evaluation. *J Hepatol*. 2016;64:1033–1040.
65. Lenggenhager D, Weber A. Hepatitis E virus and the liver: clinical settings and liver pathology. *Gastroenterol Clin North Am*. 2017;46:393–407.
66. Gupta P, Jagya N, Pabhu SB, et al. Immunohistochemistry for the diagnosis of hepatitis E virus infection. *J Viral Hepat*. 2012;19:e177–e183.
67. Pischke S, Hartl J, Pas SD, et al. Hepatitis E virus: infection beyond the liver? *J Hepatol*. 2017;66:1082–1095.
68. Perrin HB, Cintas P, Abravanel F, et al. Neurologic disorders in immunocompetent patients with autochthonous acute hepatitis E. *Emerg Infect Dis*. 2015;21:1928–1934.
69. van Eijk JJJ, Dalton HR, Ripellino P, et al. Clinical phenotype and outcome of hepatitis E virus-associated neuralgic amyotrophy. *Neurology*. 2017;89:909–917.
70. Dalton HR, Kamar N, van Eijk JJ, et al. Hepatitis E virus and neurological injury. *Nat Rev Neurol*. 2016;12:77–85.
71. Dalton HR, van Eijk JJJ, Cintas P, et al. Hepatitis E virus infection and acute non-traumatic neurological injury: a prospective multicentre study. *J Hepatol*. 2017;67:925–932.
72. Woolson KL, Forbes A, Vine L, et al. Extra-hepatic manifestations of autochthonous hepatitis E infection. *Aliment Pharmacol Ther*. 2014;40:1282–1291.
73. Dalton HR, Seghatchian J. Hepatitis E virus: emerging from the shadows in developed countries. *Transfus Apher Sci*. 2016;55: 271–274.
74. Dalton HR, Webb GW, Norton BC, et al. Hepatitis E virus: time to change the textbooks. *Dig Dis*. 2016;34:308–316.
75. Adlhoch C, Avellon A, Baylis SA, et al. Hepatitis E virus: assessment of the epidemiological situation in humans in Europe, 2014/15. *J Clin Virol*. 2016;82:9–16.
76. Teo CG. 19th-century and early 20th-century jaundice outbreaks, the USA. *Epidemiol Infect*. 2018;146:138–146.
77. Kuniholm MH, Engle RE, Purcell RH, et al. Hepatitis E virus seroprevalence in the United States: no easy answers. *Hepatology*. 2015;61:1441–1442.
78. Ditah I, Ditah F, Devaki P, et al. Current epidemiology of hepatitis E virus infection in the United States: low seroprevalence in the National Health and Nutrition Evaluation Survey. *Hepatology*. 2014;60:815–822.